# Kidney Xenotransplantation
## Steps toward Clinical Application

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## Introduction

Xenotransplantation holds the promise of turning a scarce resource, viable solid organ allografts, into a commodity that can be procured *en masse* for all individuals who would benefit. Although living donor donation has alleviated some of the problem, the scale of ESKD dwarfs any efforts to reduce scarcity on the basis of this solution alone. Additionally, risks to living donors are not insignificant, with multiple studies identifying a 2.5% incidence of major complications and higher rates of CKD and ESKD, especially among black donors (1). It is imperative to increase the supply of organs because transplantation has consistently been shown to have superior mortality, morbidity, and economic outcomes when compared with dialysis.

One pathway toward increasing this supply is xenotransplantation. However, despite substantial preclinical progress, many challenges remain in advancing this modality to the clinic. These include (*1*) finding an appropriate immunosuppressive regimen, (*2*) determining appropriate genetic modifications for minimum immunogenicity and maximum donor animal health, (*3*) defining evidence-based goals for animal models to trigger human clinical trials, and (*4*) identifying appropriate clinical cohorts for initial trials.

## Immunosuppression: The Search for a Clinically Relevant Regimen

Although myriad trials of xenotransplantation in large animal models have been performed, essentially all of these have relied on immunosuppressive regimens that are more intensive than those used in allotransplantation. Currently, as many as 40% of kidney allografts are lost because of recipient death with a functioning graft. Much of this is attributable to the complications of calcineurin inhibitors (2). Indeed, there is little evidence that humans will tolerate substantially more aggressive chronic maintenance immunosuppression than is already characteristic of allotransplantation.

Essentially all preclinical transplant models have utilized drugs that are not available in humans, including blockade of the CD154/CD40 pathway, used for its unique and highly effective immunosuppressive properties. However, early enthusiasm in human trials was dampened because of thrombotic complications. In a recent review of contemporary immunosuppression in animal models of xenotransplantation, all but one of the published studies in kidney xenotransplantation and >80% of published studies across all xenotransplanted organs utilized a CD154/CD40 blockade-based immunosuppressive regimen (3). Given this ubiquity, the ability to block this pathway may need to be translated to humans before meaningful clinical trials can be performed. Although trials are emerging in autoimmunity suggesting that CD154-specific agents may be available in the future (4), at present there is no evidence of enthusiasm for development of these agents specifically for xenotransplantation. Thus, we remain challenged to find tolerable regimens that use clinically available agents.

## Promise and Practicalities of Genetic Modification

As noted above, there are multiple difficulties with immunosuppression in xenotransplantation. One mechanism by which immunosuppression may be minimized is through the deletion of clinically relevant xenoantigens. Some of the most elegant experiments in large animal models have shown that the viability of xenotransplanted organs can be enhanced *via* genetic modifications. Recently, investigators have developed pigs that do not express the three most highly antigenic carbohydrates that have been identified: galactose-$\alpha$ 1,3 galactose, *N*-glycolylneuraminic acid, and Sd(a) antigen. Additionally, there have been efforts to "knock-in" modifications that would allow for the regulation of human complement by porcine cells as well as the regulation of coagulation cascades (5).

Still, there is much work to be done in these models. First, these deleted glycans only represent a small (albeit important) subset of all xenoantigens. Continued systematic investigation into highly immunogenic antigens will be needed to decrease the amount of immunosuppression necessary for xenotransplantation. Additionally, hurdles in regard to coagulopathy are especially important, considering the extreme sensitivity of kidneys to changes in perfusion and the associated kidney disease, including hypertension, diabetes mellitus, lupus nephritis, Henoch–Schönlein syndrome, and sickle cell disease, to name a few.

  

Two additional aspects of xenotransplantation deserve mention. First, there are little data on the off-target effects of contemporary gene editing systems such as CRISPR/Cas9, which could have variable and deleterious effects on xenograft donor viability or graft function (6). More immediately, few authors publish data on the overall health of their genetically modified pigs. However, anecdotal evidence suggests that as the number of genetic modifications increases, pigs become generally sicker and the efficiency of successfully creating, implanting, and gestating embryos decreases. Therefore, paradoxically, it may be the supply of xenograft organs that limits continued experimentation and their translation to the clinic.

## Defining a Bar for Large Animal Studies

Given that preclinical work has now moved to a number of large animal models, it is imperative to set an evidence-based goal for large animal models of xenotransplantation that would be considered sufficient to warrant studies in humans. First, it must be noted that enhancements in dialysis access and medical management of the sequelae of ESKD have led to improved outcomes for all patients with ESKD. Additionally, progress in desensitization regimens and the ability to transplant individuals with a positive crossmatch means that the burden of proof for xenotransplantation is only increasing.

Given these improvements in treatment options in patients with kidney disease, it will be difficult for xenotransplantation to achieve equipoise with our currently available treatments. One way of conceptualizing of the problem would be to liken the immunologic difference between a genetically modified pig and a human to that of a maximally HLA-mismatched individual, where the highest survival we can expect is approximately 80% at 5 years. Given the cohort of patients likely to receive initial xenotransplants (see below), a reasonable comparison may actually be highly sensitized individuals. In these individuals with a positive crossmatch, 5-year patient survival was approximately 65% (7). Finally, all therapies must be considered in relationship to overall survival on dialysis, which is approximately 50% at 5 years. To merit human clinical trials, large animal models of xenotransplantation must at least confer survival that is reliably noninferior to dialysis, approximately 90% at 1 year.

## Early Indications for Transplantation

As stated, one of the largest barriers to xenotransplantation is the relative efficacy of currently existing kidney replacement therapies. For early clinical studies to be carried out with any degree of equipoise, they will need to be conducted in individuals for whom alternative methods of kidney replacement are either exhausted or inferior. Recently, four general groups that may be initially amenable to xenotransplantation were proposed: (*1*) patients with rapidly recurrent kidney diseases such as FSGS and membranoproliferative GN type 2, (*2*) patients that are highly allosensitized, (*3*) individuals on dialysis who no longer have functioning dialysis access, and (*4*) elderly patients without significant disease in which a shorter lived graft may be appropriate (8).

This means that the initial group of patients that will undergo xenotransplantation may be less healthy than average. We believe this is appropriate. Given their relative debility, xenotransplant may actually extend these patients' lives. Therefore, the comparator will not be an individual eligible for living donor kidney transplant, but rather the secular lifespan of individuals with comparable disease.

## Infectious Concerns

An additional concern of xenotransplantation is that of zoonotic transmission of infectious diseases to both the recipient and to human populations more broadly. Although contemporary pathogen-free facilities and the development of extensive testing have ameliorated some concerns, questions still remain regarding porcine endogenous retroviruses (PERVs), which are integrated into the pig genome and are therefore present within all transplanted tissues. There have been no documented transmissions of PERVs to humans who have received porcine islet transplants or been crossperfused with *ex vivo* pig livers. Additionally, there is emerging evidence that PERVs can be eliminated through gene editing techniques (9), and that these retroviruses would be controlled by currently available antiretrovirals (10). Therefore, the risk of infectious complications from xenotransplantation appears to be similarly mitigatable compared with allotransplantation.

## A Path Forward

Although many barriers remain, the opportunities of xenotransplantation are great. To address the above challenges, we propose the following solutions.

First, issues of immunosuppression must gain more attention in the xenotransplantation literature. As the majority of the morbidity associated with contemporary allotransplantation is related to aggressive immunosuppression, more care must be taken to develop and test less intensive and clinically available immunosuppressive strategies in animal models. Second, although genetic modifications are an attractive way to decrease immunogenicity, practical considerations abound regarding both reproductive capacity and overall animal health. Xenotransplantation groups must publish more extensively on the realities of producing these animals and elucidate a research plan to address these issues. Third, an objective bar for success in large animal models that would warrant trials in humans must be developed. We propose that the dialysis survival curve for highly sensitized individuals, with a reliable 1-year survival of 90%, could be such a goal. Finally, we must define groups that are marginal candidates for convention kidney replacement and therefore be amenable to xenotransplantation. Creation of a tracked registry of these patients would allow them to serve as historical controls and expedite eventual human studies of xenotransplantation. With the substantial progress that has been made in the past decade, clinical application becomes more reasonable to contemplate. As such, defining the criteria that will allow a rational move into the clinic is increasingly important.

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**Disclosures**

None.

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